

201-14159B

HUMAN HEALTH ENDPOINTS

15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)

Source: Cardolite Corporation

Lot No.: LP-2

Composition: 78% cardanol, 8% cardol, 2% polymeric material, < 1% 2-methyl cardanol, 2.3% heptadecyl homologue triene, 3.8% heptadecyl homologue diene, 5.04% other homologous phenols

METHOD

- Method: OECD 471
- Test Type: Reverse Mutation Assay (Ames Test)
- System of testing: Bacterial
- GLP: Yes
- Year: 1995
- Species/Strain: Salmonella typhimurium (TA1535, TA1537, TA1538, TA98 & TA100)
- Metabolic activation: S9-mix, Rat liver cells, 0.5 ml, Aroclor induced
- Concentrations tested: 50, 150, 500, 1500, 5000 µg/plate (\pm S9)
- Statistical Methods: Dunnett's method of linear regression

Remarks:

- Test Design

- Number of replicates: 3
- Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine (-S9, TA100 & TA1535)
9-aminoacridine (-S9, TA 1537)
4-nitro-o-phenylenediamine (-S9, TA1538)
4-nitroquinoline-1-oxide (-S9, TA98)
2-aminoanthracene (+S9, TA98, TA100, TA1535, TA1537 & TA1538)
- Negative control: Solvent vehicle

- Solvent: Acetone

RESULTS

- Result: Negative
- Cytotoxic concentration
 - With metabolic activation: > 5000 µg/plate
 - Without metabolic activation: > 5000 µg/plate
- Genotoxic effects
 - With metabolic activation: None
 - Without metabolic activation: None

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- Statistical results:** No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation.

Experiment 1 – Without Metabolic Activation

Test Substance Concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	115	28	34	36	10
	117 (110)	25 (21)	25 (25)	30 (30)	17 (15)
	97 11.0	9 10.2	17 8.5	25 5.5	18 4.4
50	149	20	17	17	19
	118 (131)	19 (19)	27 (23)	24 (21)	18 (18)
	127 15.9	18 1.0	24 5.1	23 3.8	18 0.6
150	118	12	9	24	15
	120 (120)	10 (12)	28 (16)	17 (18)	18 (17)
	121 1.5	15 2.5	11 10.4	14 5.1	18 1.7
500	121	17	13	17	10
	115 (116)	20 (16)	30 (18)	33 (25)	12 (12)
	111 5.0	12 4.0	12 10.1	25 8.0	14 2.0
1500	115p	8p	15p	22p	13p
	107p (117)	22p (16)	17p (15)	25p (27)	10p (12)
	128p 10.6	17p 7.1	14p 1.5	34p 6.2	13p 1.7
5000	122p	7p	25p	29p	12p
	85p (106)	15p (10)	15p (19)	22p (23)	19p (16)
	111p 19.0	8p 4.4	18p 5.1	17p 6.0	18p 3.8
Positive Control	ENNG	ENNG	4NOPD	4NQO	9AA
Concentration ($\mu\text{g}/\text{plate}$)	3	5	5	0.2	80
Number of colonies per plate	670	198	470	168	76
	933 (729)	213 (224)	474 (479)	152 (156)	208 (152)
	583 182.2	260 32.3	494 12.9	149 10.2	172 68.2

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.
 Positive controls: ENNG (N-ethyl-N'-nitro-N-nitroguanidine), 4NOPD (4-nitro- σ -phenylenediamine), 4NQO (4-nitroquinoline-1-oxide), 9AA (9-aminoacridine)

Experiment 1 – With Metabolic Activation					
Test Substance Concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	131	19	39	24	17
	108 (113)	17 (17)	35 (33)	38 (32)	18 (16)
	101 15.7	15 2.0	24 7.8	35 7.4	14 2.1
50	118	18	34	29	17
	129 (125)	13 (15)	24 (32)	34 (33)	13 (16)
	127 5.9	15 2.5	39 7.6	35 3.2	17 2.3

	121	13	32	33	10
150	111 (117)	19 (16)	33 (33)	33 (35)	15 (15)
	120 5.5	17 3.1	33 0.6	39 3.5	19 4.5
	111	10	18	35	12
500	143 (115)	15 (15)	35 (29)	38 (33)	15 (12)
	91 26.2	19 4.5	34 9.5	27 5.7	10 2.5
	98p	20p	25p	28p	13p
1500	133p (114)	17p (19)	28p (27)	28p (30)	18p (15)
	112p 17.6	20p 1.7	28p 1.7	33p 2.9	13p 2.9
	128p	12p	30p	29p	14p
5000	112p (117)	17p (14)	18p (24)	28p (29)	19p (17)
	112p 9.2	13p 2.6	25p 6.0	30p 1.0	17p 2.5
Positive Control	2AA	2AA	2AA	2AA	2AA
Concentration ($\mu\text{g}/\text{plate}$)	1	2	0.5	0.5	2
Number of colonies per plate	1332	64	353	219	194
	1615(1382)	69 (66)	323 (361)	453 (329)	252 (232)
	1200 212.0	64 2.9	408 43.1	315 117.6	250 32.9

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive control: 2AA (2-aminoanthracene)

Experiment 2 – Without Metabolic Activation					
Test Substance Concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	107	14	29	17	19
	132 (116)	18 (17)	22 (21)	23 (21)	19 (17)
	110 13.7	19 2.6	12 8.5	24 3.8	13 3.5
50	149	12	41	34	10
	133 (140)	18 (21)	22 (29)	30 (32)	20 (14)
	138 8.2	32 10.3	23 10.7	0	13 5.1
150	133	18	10	35	14
	118 (129)	24 (24)	22 (17)	33 (32)	10 (12)
	137 10.0	29 5.5	19 6.2	28 3.6	12 2.0
500	134	13	20	23	13
	139 (131)	13 (15)	14 (21)	34 (25)	19 (19)
	121 9.3	20 4.0	30 8.1	19 7.8	24 5.5
1500	117p	12p	22p	17p	18p
	117p (109)	10p (12)	23p (21)	20p (26)	12p (15)
	92p 14.4	14p 2.0	18p	40p 12.5	14p 3.1
5000	107p	10p	19p	20p	14p
	121p (108)	25p (19)	19p (20)	24p (28)	12p (12)
	95p 13.0	23p 8.1	23p 2.3	39p 10.0	10p 2.0
Positive Control	ENNG	ENNG	4NOPD	4NQO	9AA
Concentration ($\mu\text{g}/\text{plate}$)	3	5	5	0.2	80
Number of colonies per plate	916	514	406	177	638
	711 (711)	504 (498)	499 (455)	203 (196)	656 (589)
	740 110.9	477 19.1	459 46.7	208 16.6	474 100.3

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.
Positive controls: ENNG (N-ethyl-N'-nitro-N-nitroguanidine), 4NOPD (4-nitro- σ -phenylenediamine), 4NQO (4-nitroquinoline-1-oxide), 9AA (9-aminoacridine)

Experiment 2 – With Metabolic Activation						
Test Substance Concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (Number of colonies per plate)					
	Base-pair substitution type		Frameshift type			
	TA100	TA1535	TA1538	TA98	TA1537	
0	137	25	35	24	22	
	139 (131)	20 (20)	28 (34)	28 (32)	18 (18)	
	117 12.2	15 5.0	39 5.6	44 10.6	13 4.5	
50	133	24	31	38	19	
	138 (128)	22 (21)	33 (32)	33 (34)	24 (19)	
	112 13.8	18 3.1	32 1.0	32 3.2	13 5.5	
150	108	23	25	29	14	
	120 (115)	30 (25)	35 (34)	36 (33)	22 (17)	
	118 6.4	22 4.4	43 9.0	35 3.8	14 4.6	
500	122	23	23	28	13	
	142 (125)	24 (23)	30 (26)	24 (32)	13 (16)	
	110 16.2	23 0.6	25 3.6	44 10.6	22 5.2	
1500	129p	13p	25p	28p	17p	
	120p (123)	15p (19)	22p (25)	13p (26)	17p (16)	
	121p 4.9	30p 9.3	28p 3.0	36p 11.7	14p 1.7	
5000	128p	18p	32p	36p	15p	
	170p (135)	20p (18)	17p (29)	35p (36)	15p (15)	
	106p 32.5	15p 2.5	38p 10.8	36p 0.6	14p 0.6	
Positive Control	2AA	2AA	2AA	2AA	2AA	
Concentration ($\mu\text{g}/\text{plate}$)	1	2	0.5	0.5	2	
Number of colonies per plate	1398	102	276	159	255	
	1553(1406)	139 (114)	256 (273)	293 (243)	250 (254)	
	1268 142.7	102 21.4	286 15.3	278 73.4	258 4.0	

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.
Positive Control: 2AA (2-aminoanthracene)

Remarks: A precipitate was observed at and above 1500 $\mu\text{g}/\text{plate}$, however this did not interfere with the scoring of revertant colonies.

CONCLUSIONS

Remarks: The substance was found to be non-mutagenic under the conditions of the test.

DATA QUALITY

- Reliabilities: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: Reverse Mutation Assay 'Ames Test' Using Salmonella Typhimurium, Report No. 814/001, 1995

Kirkland, D.J., (Ed), Statistical Evaluation of Mutagenicity Test Data, UKEMS Sub-committee on Guidelines for Mutagenicity Testing, Report - Part III (1989), Cambridge University Press

OTHER

- **Last Changed:** 17 December 2002
- **Order number for sorting:** 1

Remarks:

HUMAN HEALTH ENDPOINTS

15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)

Source: Cardolite Corporation

Lot No.: LP-2

Composition: 78% cardanol, 8% cardol, 2% polymeric material, < 1% 2- methyl cardanol, 2.3% heptadecyl homologue triene, 3.8% heptadecyl homologue diene, 5.04% other homologous phenols

METHOD

- Method:** OECD 473
- Test Type:** Chromosomal aberration test
- System of testing:** Non bacterial
- GLP:** Yes
- Year:** 1995
- Species/Strain:** Human Lymphocytes
- Metabolic activation:** S9-mix, Rat liver cells, Aroclor induced, 1 ml
- Concentrations tested:**
 - Expt. 1 (20h harvest): 0, 6.25, 12.5, 25 µg/ml (-S9)
0, 3.125, 6.25, 12.5 µg/ml (+S9)
 - Expt. 2 (20h harvest): 12.5, 25, 37.5 µg/ml (-S9)
0.78, 1.56, 3.125, µg/ml (+S9)
 - Expt. 2 (44h harvest): 25 µg/ml (-S9)
3.125 µg/ml (+S9)
- Statistical Methods:** Fisher's Exact test or Chi-squared test

Remarks:

- Test Design

- Number of replicates:** 2
- Positive control:** Ethyl methanesulphonate (EMS) (-S9), cyclophosphamide (+S9)
- Negative control:** Solvent vehicle

- Solvent: Dimethylsulfoxide

RESULTS

- Result:** Negative
- Cytotoxic concentration**
 - With metabolic activation:** 12.5 µg/ml
 - Without metabolic activation:** >25 µg/ml
- Genotoxic effects**

- **With metabolic activation:** None
- **Without metabolic activation:** None
- **Statistical results:** The test material did not induce a significant increase in the frequency of cells with chromosome aberrations or polyploid cells in either the presence or absence of a liver enzyme metabolizing system.

Experiment 1: Harvest Time 20 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others X	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges		(+ Gaps)	(-Gaps)	(+ Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	1	0	0	0	1	1	1	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	0	0	1	0	0	0	1	1	1	1
			(0.0)	(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
6.25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
12.5 µg/ml	A	100	2	1	0	0	0	0	3	1	3	1
	B	100	0	0	0	1	0	0	1	1	1	1
	Total	200	2	1	0	1	0	0	4	2	4	2
			(1.0)	(0.5)	(0.0)	(0.5)	(0.0)	(0.0)	(2.0)	(1.0)	(2.0)	(1.0)
25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	1	1	0	0	0	0	2	1	2	1
	Total	200	2	1	0	0	0	0	3	1	3	1
			(1.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(0.5)	(1.5)	(0.5)
Positive Control EMS 500 µg/ml	A	50	31	14	7	2	1	0	55	24	33	21
	B	50	13	18	8	2	0	0	41	28	29	24
	Total	100	44	32	15	4	1	0	96	52	62***	45***
			(44.0)	(32.0)	(15.0)	(4.0)	(1.0)	(0.0)	(96.0)	(52.0)	(62.0)	(45.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells *** represents p ≤ 0.001

Experiment 1: Harvest Time 20 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others X	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges		(+ Gaps)	(-Gaps)	(+ Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	0	1	0	0	0	0	1	1	1	1
			(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
1.56 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	0	1	0	0	0	0	1	1	1	1
			(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
3.125 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	1	0	0	0	0	0	1	0	1	0
	Total	200	2	0	0	0	0	0	2	0	2	0

			(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.0)
6.25 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	4	0	0	0	0	0	4	0	4	0
	Total	200	4	0	0	0	0	0	4	0	4	0
			(2.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(2.0)	(0.0)	(2.0)	(0.0)
Positive Control CP 25 µg/ml	A	100	4	0	0	1	0	0	5	1	5	1
	B	100	1	2	0	2	0	0	5	4	4	3
	Total	200	5	2	0	3	0	0	10	5	9**	4
			(2.5)	(1.0)	(0.0)	(1.5)	(0.0)	(0.0)	(5.0)	(2.5)	(4.5)	(2.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells ** represents p ≤ 0.01

Experiment 2: Harvest Time 20 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others X	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges		(+ Gaps)	(-Gaps)	(+ Gaps)	(-Gaps)
Vehicle Control	A	100	2	0	0	0	0	0	2	0	2	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	2	0	0	0	0	0	2	0	2	0
			(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.0)
12.5 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
25 µg/ml	A	100	1	1	0	0	0	0	2	1	2	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	1	0	0	0	0	2	1	2	1
			(0.5)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
37.5 µg/ml	A	100	1	1	0	0	0	0	2	1	2	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	1	0	0	0	0	2	1	2	1
			(0.5)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
Positive Control EMS 500 µg/ml	A	100	6	9	4	0	0	0	19	13	13	11
	B	100	16	17	2	1	0	0	36	20	26	15
	Total	200	22	26	6	1	0	0	55	33	39***	26***
			(11.0)	(13.0)	(3.0)	(0.5)	(0.0)	(0.0)	(27.5)	(16.5)	(19.5)	(13.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells *** represents p ≤ 0.001

Experiment 2: Harvest Time 20 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others X	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges		(+ Gaps)	(-Gaps)	(+ Gaps)	(-Gaps)
Vehicle Control	A	100	1	1	1	0	0	0	3	2	3	2
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	1	2	1	0	0	0	4	3	4	3
			(0.5)	(1.0)	(0.5)	(0.0)	(0.0)	(0.0)	(2.0)	(1.5)	(2.0)	(1.5)
0.78 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	3	0	0	0	0	3	3	3	3
	Total	200	0	3	0	0	0	0	3	3	3	3

			(0.0)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)	(1.5)
1.56 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0	
	B	100	0	0	0	0	0	0	0	0	0	0	
	Total	200	1	0	0	0	0	0	1	0	1	0	
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)	
3.125 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0	
	B	100	1	0	0	0	1	0	2	1	2	1	
	Total	200	1	0	0	0	1	0	2	1	2	1	
			(0.5)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)	
Positive Control CP 25 µg/ml	A	100	5	4	0	1	0	0	10	5	9	5	
	B	100	6	0	2	1	0	0	9	3	8	3	
	Total	200	11	4	2	2	0	0	19	8	17**	8	
			(5.5)	(2.0)	(1.0)	(1.0)	(0.0)	(0.0)	(9.5)	(4.0)	(8.5)	(4.0)	

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells ** represents p ≤ 0.01

Experiment 2: Harvest Time 44 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others X	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges		(+ Gaps)	(-Gaps)	(+ Gaps)	(-Gaps)
Vehicle Control	A	100	0	3	0	0	0	0	3	3	3	3
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	0	3	0	0	0	0	3	3	3	3
			(0.0)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)

X = > 10 aberrations per cell (not included in total aberrations)

Figures in brackets = aberrations per 100 cells

Experiment 2: Harvest Time 44 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others X	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges		(+ Gaps)	(-Gaps)	(+ Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	0	1	0	0	1	1	1	1
	B	100	0	1	0	1	0	0	2	2	2	2
	Total	200	0	1	0	2	0	0	3	3	3	3
			(0.0)	(0.5)	(0.0)	(1.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
25 µg/ml	A	100	2	1	0	1	0	0	4	2	4	2
	B	100	1	0	0	0	0	0	1	0	1	0
	Total	200	3	1	0	1	0	0	5	2	5	2
			(1.5)	(0.5)	(0.0)	(0.5)	(0.0)	(0.0)	(2.5)	(1.0)	(2.5)	(1.0)

X = > 10 aberrations per cell (not included in total aberrations)

Figures in brackets = aberrations per 100 cells

Experiment 1: Mean Frequency of Polyploid Cells (%)

Dose Level µg/ml	20 Hours	
	Without S9	With S9
0	0.0	0.0
1.56	-	0.5

3.125	-	0.0
6.25	0.0	0.0
12.5	0.0	-
25	0.0	-
EMS 500	0.0	-
CP 25	-	0.0

Experiment 2: Mean Frequency of Polyploid Cells (%)

Dose Level μg/ml	Without S9		Dose Level μg/ml	With S9	
	20 hours	44 hours		20 hours	44 hours
0	0.0	0.5	0	0.0	1.0
12.5	0.5	-	0.78	0.0	-
25	0.0	0.5	1.56	1.0	-
37.5	0.0	-	3.125	1.0	0.0
EMS 500	0.0	-	CP 25	0.5	-

Remarks:

Experiment 1: Mitotic Index (20-hour harvest)

Dose Level μg/ml	Without S9				With S9			
	A	B	Mean	% of Control	A	B	Mean	% of Control
0	5.80	6.25	6.03	100	3.10	2.40	2.75	100
0.78					-	-	-	-
1.56	-	-	-	-	-	-	-	-
3.125	-	-	-	-	3.60	3.60	3.60	131
6.25	4.90	7.80	6.35	105	1.15	2.25	1.70	62
12.5	6.70	6.50	6.60	109	0.85	0.55	0.70	25
25	8.30	4.30	6.30	104	-	-	-	-
50	NM	NM	-	-				
EMS 500	3.40	4.30	3.85	64				
CP 25	-	-	-	-	1.40	2.45	1.93	70

- = not assessed

NM = no scorable metaphases

Experiment 2: Mitotic Index (20-hour harvest)

Dose Level μg/ml	Without S9				With S9			
	A	B	Mean	% of Control	A	B	Mean	% of Control
0	8.55	7.90	8.23	100	3.00	3.25	3.13	100
0.39					-	-	-	-
0.78					1.80	3.35	2.58	82
1.56					2.50	2.80	2.65	85
3.125	-	-	-	-	1.35	1.90	1.63	52
6.25	7.20	6.75	6.98	85	0.45	0.45	0.45	14
9.38					NM	NM	-	-
12.5	7.75	9.45	8.60	104				

25	6.00	9.45	7.73	94					
37.5	3.25	3.65	3.45	42					
50	NM	NM	NM	-					
EMS 500	4.70	7.95	4.83	59	-	-	-	-	
CP 25	-	-	-	-	1.60	1.45	1.53	49	

- = not assessed NM = no scorable metaphases

CONCLUSIONS

Remarks: The substance was found to be non -clastogenic under the conditions of the test.

DATA QUALITY

- Reliabilities: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: Chromosome Aberration Test in Human Lymphocytes In Vitro, Report No. 814/002, 1995

Scott, D., Et al, Metaphase chromosome aberration assays in vitro. In: Kirkland, D.J., Basic mutagenicity tests: UKEMS recommended procedures. Report. Part 1 revised. Cambridge University Press, 1990:62-84

OTHER

- Last Changed: 17 December 2002
- Order number for sorting: 2

Remarks:

HUMAN HEALTH ENDPOINTS

15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)

Source: Cardolite Corporation

Lot No.: LP-2

Composition: 78% cardanol, 8% cardol, 2% polymeric material, < 1% 2-methyl cardanol, 2.3% heptadecyl homologue triene, 3.8% heptadecyl homologue diene, 5.04% other homologous phenols

METHOD

- Method: OECD 476
- Test Type: Forward Mutation Assay
- System of testing: Non bacterial
- GLP: Yes
- Year: 1996
- Species/Strain: Chinese Hamster Ovary CHO-KI BH4
- Metabolic activation: S9-mix, Rat liver cells, Aroclor induced
- Concentrations tested:

Expt. 1:	0, 0.75, 1.5, 3, 6, 12 µg/ml (-S9)
	0, 1.5, 3, 6, 12, 18 µg/ml (+S9)
Expt. 2:	0, 0.75, 1.5, 3, 6, 9 µg/ml (-S9)
	0, 3, 6, 12, 18, 24 µg/ml (+S9)
- Statistical Methods: Cochran-Armitage test for trend analysis, Fisher-Irwin exact test for group comparisons for proportions.

Remarks:

- Test Design

- Number of replicates: 2
 - Positive control: Ethyl methanesulphonate (EMS) (-S9), 3-methylcholanthrene (3-MC) (+S9)
 - Negative control: Solvent vehicle
- Solvent: Dimethylsulfoxide

RESULTS

- Result: Negative
- Cytotoxic concentration
 - With metabolic activation: 47.19 µg/ml
 - Without metabolic activation: 47.19 µg/ml
- Genotoxic effects
 - With metabolic activation: None

- **Without metabolic activation:** None
- * **Statistical results:** The test material did not induce significant or dose-related increases in mutant frequency per survivor in either the presence or absence of metabolic activation in either of the two experiments.

Summary of Results:

Experiment 1:

Dose Level µg/ml	Without S9		Mean MFS	Dose Level µg/ml	With S9		Mean MFS
	A	B			A	B	
0	3.4	0.7	2.05	0	3.5	3.3	3.4
0.75	1.4	-	1.4	1.5	2.9	0.7	1.80
1.5	2.0	0.0	1.00	3.0	0.6	1.4	1.00
3	0.0	0.0	0.0	6.0	2.9	0.0	1.45
6	0.0	0.0	0.0	12	1.4	6.3	3.85
12	0.0	6.3	3.15	18	0.7	8.6	4.65
EMS 200	154.5	189.9	172.20	24	-	-	-
				3-MC 4	238.8	285.9	262.35

MFS = 6-TG resistant mutants/10⁶ viable cells

Experiment 2:

Dose Level µg/ml	Without S9		Mean MFS	Dose Level µg/ml	With S9		Mean MFS
	A	B			A	B	
0	0.0	0.6	0.30	0	8.1	0.8	4.45
0.75	0.4	7.6	4.00	3	1.3	0.0	0.65
1.5	3.2	0.9	2.05	6	0.9	0.0	0.45
3	0.6	6.2	3.40	2	0.0	0.0	0.00
6	0.5	1.7	1.10	18	0.0	0.0	0.00
9	0.6	0.0	0.30	24	TOXIC		
EMS 200	158.3	149.1	153.70				
				3-MC 4	284.6	278.1	281.35

MFS = 6-TG resistant mutants/10⁶ viable cells

Remarks:

CONCLUSIONS

Remarks: The test material was found to be non-mutagenic to CHO cells at the HGPRT locus under the conditions of this test.

DATA QUALITY

- * **Reliabilities:** 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: CHO HGPRT Forward Mutation Assay, Report No. 814/003, 1996

Cole, J., et al, (1990): Gene Mutation in Cultured Mammalian Cells. In 'Basic Mutagenicity Tests: UKEMS Recommended Procedures', (ed D.J. Kirkland), Cambridge University Press, New York,

OTHER

- * **Last Changed:** 19 December 2002
- * **Order number for sorting:** 3

Remarks:

ENVIRONMENTAL FATE ELEMENTS AND PATHWAYS

9. BIODEGRADATION

TEST SUBSTANCE

- **Identity:** Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)

Source: Cardolite Corporation

Lot No.: LP-2

Composition: 78% cardanol, 8% cardol, 2% polymeric material, < 1% 2-methyl cardanol, 2.3% heptadecyl homologue triene, 3.8% heptadecyl homologue diene, 5.04% other homologous phenols

METHOD

- **Method:** OECD Method 302D
- **Test Type:** aerobic
- **GLP:** Yes
- **Year:** 1993
- **Contact time:** 28 (days)
- **Innoculum:** activated sludge

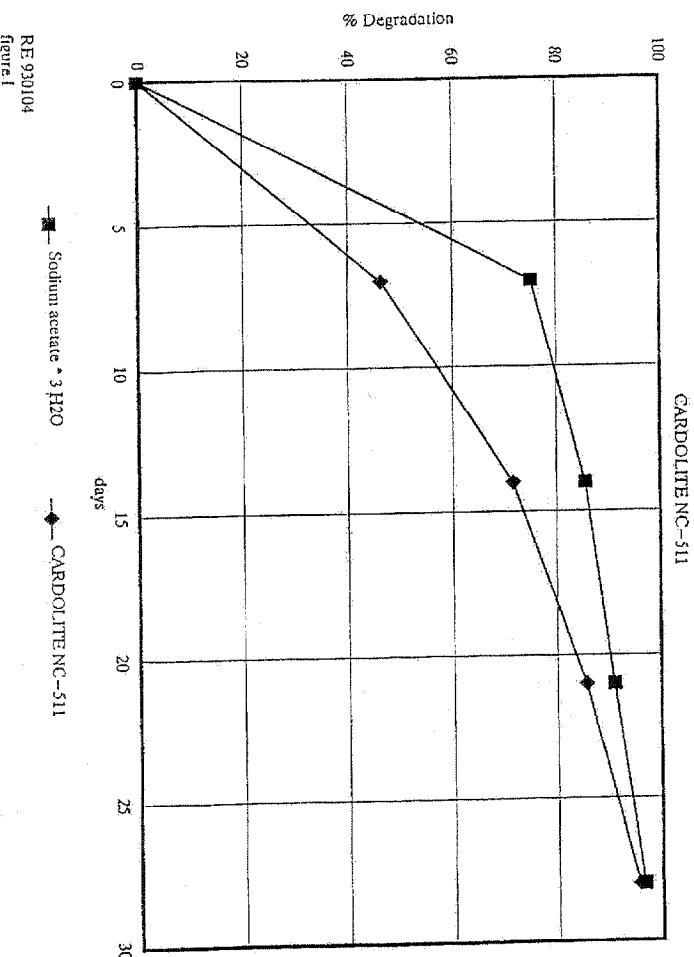
Remarks:

- **Innoculum:** Fresh activated sludge from a municipal biological sewage treatment plant. 30 mg suspended solids/l of test medium.
- **Concentration of test chemical:** 6.01 – 6.39 mg, direct addition
- **Temperature of incubation:** 20°C
- **Preacclimation:** None
- **Dosing procedure:** Test substance weighed on a piece of glass to an amount of about 20 mg ThOD (or COD) and added directly to the test flask.
- **Sampling frequency:** 0,7,14,21 & 28 days
- **Controls:** Sodium acetate used as positive control, inoculum used as blank.
- **Analytical method used to measure biodegradation:** The COD of the poorly soluble substance was determined in a variation of ISO Method 6060 (closed system with a pressure equaliser / Kelkenberg method, Z.f. Wasser und Abwasserforschung (1975) 146). Oxygen determination was performed using an oxygen electrode (WTW;FRG; Microprocessor oximeter OXI 2000 with electrode model TriOxmatic EO 200).
- **Method of calculating measured concentrations:** Arithmetic mean

RESULTS

- **Degradation % after time:** 96% after 28 days
- **Results:** Readily biodegradable
- **Kinetic:**

Day	% Degradation	
	Sample	Positive control
7	46	75
14	72	86
21	86	91
28	96	97



* Breakdown products: Not determined

Remarks: None

CONCLUSIONS

Remarks:

RE 930104
figure 1

According to the author of the study, based on the data (i.e. 96% degradation after 28 days) Cardolite NC-511 can be regarded as very highly biodegradable.

DATA QUALITY

- **Reliabilities:** 1, Reliable without restriction

Remarks: Study conducted under GLP to recognised test method by Henkel KGaA

REFERENCES

Henkel KGaA, Cardolite NC-511 Ultimate biodegradability in the BODIS -Test, Report No. RE930104, 1993

OTHER

Last Changed: 17 December 2002

Order number for sorting: 1

Remarks:

The test method used was based on the closed bottle test (OECD test method 302D) and the RDA-Blok test, previously published (Blok, J., A Repetitive Die Away (RDA) Test Combining Several Biodegradability Test Procedures, Int. Biodeterior. Bull., 15 (1979) 57-63) and ring -tested by the OECD (1988 ring test on ready biodegradability).